trafficking of Kv channels to and from the cell surface. TIRF-based studies indicated that GFP-Kv2.1 containing vesicles tether directly to and deliver cargo in a discrete fashion to the Kv2.1 surface clusters in both transfected HEK and cultured hippocampal neurons. Qdot-based single molecule experiments indicated that the delivery and surface retrieval of Kv2.1 occurs at the perimeter of the surface clusters. Overall, 85 ± 8.4% of newly synthesized channels in HEK cells and 84.9 ± 10.4% in hippocampal neurons were inserted at the cluster perimeter even though the Kv2.1 clusters represent only 21.4 ± 3.8% of the basal cell surface. When 132 continuously recycling Kv2.1 channels in HEK cells were examined, 96.2% were also inserted at the cluster perimeter. The actin depolymerizing agents swinholide A and cytochalasin D reduced the cluster localized insertion to 5 and 0% of control, respectively. Unlike Kv2.1, the Kv1.4 K+ channel has a homogeneous cell-surface expression in transfected cells. Demonstrating that Kv2.1 clusters represent cell-surface platforms for more than just Kv2.1 insertion and retrieval, the non-clustering Kv1.4 K+ channel also inserted into the HEK cell plasma membrane at the Kv2.1 cluster perimeter. Kv1.4 endocytosis also occurred at this region. Together, these results indicate that a non-conducting function of Kv2.1 is to form specialized cell-surface microdomains which are involved in ion channel trafficking. These domains may also be involved in additional cellular processes.

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Modification of Lipid Membrane Rigidity by the Yeast and Human Sar1 Vesicle Trafficking Proteins

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Understanding how proteins manipulate the shape and form of membranes is crucial to explaining key biological functions. Membrane curvature generation enables the creation of structures such as intracellular transport vesicles, and also guides geometry-dependent protein localization. We focus on Sar1, a roughly 21.5 kDa GTPase of the COPII family of coat proteins, which initiates the assembly of coated vesicles at the endoplasmic reticulum. Using optical-trap based assays involving dynamic membrane deformation, we find that the two human Sar1 proteins (Sar1a and Sar1b) dramatically lower membrane rigidity, similar to the yeast (S. cerevisiae) Sar1 previously examined. We further explore cross-linked Sar1 scaffolds that locally concentrate the protein at membrane surfaces analyzing the resulting effects on membrane bending modulus.

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Position-Dependent Effects of Poly-Lysine on Sec Protein Transport

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The bacterial Sec protein translocation system catalyzes the transport of unfolded precursor proteins across the cytoplasmic membrane. Using a recently developed real-time fluorescence-based transport assay, the effects of the number and distribution of positive charges on the transport time and transport efficiency of proOmpA were examined. As expected, an increase in the number of lysine residues generally increased transport time and decreased transport efficiency. However, the observed effects were highly dependent upon poly-lysine position in the mature domain. In addition, a string of consecutive positive charges generally had a more significant effect on transport time and efficiency than separating the charges into two or more charged segments. Thirty positive charges distributed throughout the mature domain resulted in effects similar to ten consecutive charges near the N-terminus of the mature domain. These data support a model in which the local effects of positive charge on the translocation kinetics dominate over total thermodynamic constraints. The rapid translocation kinetics of some highly charged proOmpA mutants suggest that the charge is partially shielded from the electric field gradient during transport, possibly by the co-migration of counter ions. The translocation times of precursors with multiple positively charged sequences, or ''pause sites'', were fairly well predicted by a local effect model. However, the kinetic profile predicted by this local effect model was not observed. Instead, the transport kinetics observed for precursors with multiple poly-lysine segments support a model in which translocation through the SecYEG pore is not the rate-limiting step of transport.

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Trafficking and Stability of Deafness Mutations in the Fourth Transmembrane Helix of Connexin26 Channels

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Connexins (Cx) assemble to form the intercellular communication channels in gap junctions (GJs). Mutations in Connexin26 (Cx26) that cause childhood pre-lingual deafness are found in human populations around the globe. Types of defect resulting from connexin mutations include loss of protein synthesis, mis-trafficking, hemichannel docking and defective channel function (for example, altered permeability or gating). We have characterized a new category of non-functional connexin mutations where substitutions in the transmembrane helix regions render the hexamer unstable (Ambrosi et al., (2010) Biophys. J. 98:1809).
This work on deafness mutants focuses on the fourth transmembrane helix, M4. According to the X-ray crystallographic structure of Cx26 (Maeda et al., (2009) Nature 458:597) one face of this helix sits against the lipid bilayer. Our 6 Å EM structure of the Cx26M34A mutant embedded in membrane bilayers (Oshima et al. (2011) J Mol Biol 405:724) revealed an outward expansion of the transmembrane domain relative to the X-ray crystallographic structure of the wild-type Cx26 channel in detergent. We became interested in how single site M4 amino substitutions that are distal to the channel pore, especially those that face the lipid membranes, can create non-functional channels. The 14 deafness mutants we studied in the M4 helix are not localized to a specific face of the helix and do not all cause the same type of GJ defect. Several deafness mutations in M4 cause mis-trafficking (K188R, F191L, V198M, S199F, G200R, I203K, L205P, T208P) but others (M195T, A197S, C202F, I203T, I205V, N206S) make GJs in mammalian cells. When expressed and purified from baculovirus infected Sf9 cells, C202F and N206S form hemichannels that are unstable upon detergent solubilization. Experiments are now in progress to test the functional properties of all six GJ forming mutants.

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Brownian Permeability Computation Model Predicts That Differences in the Internal Radii of the Pore are Determinant for Unidirectional and Reversal Fluxes through Gap Junction Channels

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Gap junctions are vital in multiple organs' physiology and are linked to severe genetic diseases. Connexins are the proteins forming these gap junction channels which allow molecular and ionic selective-diffusion amongst neighboring cells. Selectivity is connexin-type dependent. In between HeLa cells expressing distinct connexins (e.g. Cx43 and Cx45), heterotypic channels formed induce a preferential flux of large fluorescent molecules towards the Cx43 side. The independence to molecule's charge, points towards a high relevance of the channel's pore shape. To study the effects of pore shape on particles movement, a 3D-computational channel model was developed, emulating X-ray crystallographic structures from Cx43 and Cx26. The sections of the pore were approximated to standard geometric shapes including a central ellipsoidal vestibule. Ellipsoids were placed on either ends of the pore, representing controlled volumes from coupled cells. Particles were modeled as spheres and Brownian dynamics was used to model particles' interactions. Simulations for different pore geometries show that against conventional thought, some shapes can explain the direction of the preferential flux data that comply with the electrical resistances measured through electrophysiological recordings. This pore-geometry causes a different intra-pore mechanism that requires an asymmetric vestibule with radii comparable to the particle size. In our simulations when particles were introduced from the side of the pore where the inner mouth is larger, particles reside longer in the vestibule using it as an intermediate stop and forcing the particles to cross faster to the other side of the pore.

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Molecular Dynamics Simulations of Cx26-Wt and Deafness Related Mutants M34A, A40G and V37I

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Gap junctions are specialized regions of the cellular membrane in which protein oligomers form channels for intercellular communication that allow the exchange of nutrients, metabolites, ions and small molecules up to ~1KDa. Gap junction channels are formed by the end-to-end docking of the extracellular portion of connexins, a family of transmembrane proteins that forms hexameric arrays on the cell membrane. Mutations in at least three human connexin genes, Cx26, Cx30 and Cx31, widely expressed throughout the cochlea, are the leading causes of syndromic and nonsyndromic hereditary hearing loss. To date, the only avaliable 3D structure of this protein family corresponds to Cx26 hemi-channel. Taking this structure as a starting point we have developed fully-atomistic models of Cx26-Wt and three deafness-associated mutants: M34A, V37I and A40G. These models have been used to perform molecular dynamics simulations aimed to characterize and compare their dynamic behavior in order to get insights about the structure-function relationships coded into the molecular